cal, visual (e.g., videotape, DVD, etc.) or electronic communications (including Internet or web-based communications), provided in any manner.

[0174] In some aspects, systems and methods of promoting one or more of the embodiments described above are provided. As used herein, "promoted" includes all methods of doing business including, but not limited to, methods of selling, advertising, assigning, licensing, contracting, instructing, educating, researching, importing, exporting, negotiating, financing, loaning, trading, vending, reselling, distributing, repairing, replacing, insuring, suing, patenting, or the like that are associated with the systems, devices, apparatuses, articles, methods, compositions, kits, etc. of the invention as discussed herein. Methods of promotion can be performed by any party including, but not limited to, personal parties, businesses (public or private), partnerships, corporations, trusts, contractual or sub-contractual agencies, educational institutions such as colleges and universities, research institutions, hospitals or other clinical institutions, governmental agencies, etc. Promotional activities may include communications of any form (e.g., written, oral, and/or electronic communications, such as, but not limited to, e-mail, telephonic, Internet, Web-based, etc.) that are clearly associated with the invention.

[0175] In one set of embodiments, the method of promotion may involve one or more instructions. As used herein, "instructions" can define a component of instructional utility (e.g., directions, guides, warnings, labels, notes, FAQs or "frequently asked questions," etc.), and typically involve written instructions on or associated with the invention and/or with the packaging of the invention. Instructions can also include instructional communications in any form (e.g., oral, electronic, audible, digital, optical, visual, etc.), provided in any manner such that a user will clearly recognize that the instructions are to be associated with the invention, e.g., as discussed herein.

[0176] The following examples are intended to illustrate certain embodiments of the present invention, but do not exemplify the full scope of the invention.

EXAMPLE 1

[0177] One example illustrates a method for high-throughput screening of expressed proteins and polypeptides, according to one embodiment of the invention. Screening and directed evolution of functional proteins for new activities is still a considerable challenge. The vastness of the sequence space, i.e., the large number of possible permutations in even small proteins can make it difficult to conclude that all possible permutations were adequately tested by nature.

[0178] By using known recombinant DNA technologies, it is possible to create extremely large collections of genes, encoding mutants of a given protein. However, it has been difficult to create generic technologies that allow sampling of billions of different proteins.

[0179] Current methods to screen proteins and polypeptides for binding, catalytic or regulatory activities are based largely on screening in microtitre plates and robotic liquid handling. Today, robotic screening programs may process up to 100,000 assays a day (~1 per second). The cost of high-throughput screening is substantial, e.g., greater than $100 million. Furthermore, the reagents' costs alone are typically about a dollar per assay, putting a financial ceiling on the number off assays which can be realistically performed.

[0180] The use of screening technologies which use more inexpensive equipment and further reducing test volumes below the 1-2 microliter capacity of 3,456-well plates would create both significant cost savings and would enable higher throughput. However, using microtitre plate technology, further miniaturization can meet with some difficulties: for example, evaporation becomes more significant in microliter volumes, and capillary action can cause "wicking" and bridging of liquid between wells.

[0181] One example illustrates droplet-based microfluidics for the high-throughput screening of proteins and polypeptides for binding, catalytic, or regulatory activities. FIG. **2** summarizes this method. This system is based on performing assays in aqueous microdroplets in a carrier oil (e.g., perfluorocarbon) in a microfluidic device. Each droplet, with a typical diameter of between 10-100 micrometers (other diameters are also possible), can function as an independent microreactor, but has a volume of only ~0.5 μl to 0.5 nl (controllable by the user, depending on the size of the droplets). The volume of each assay is therefore reduced by 10^3 to 10^6-fold compared to a conventional assay in 1,536- or 3,456-well plates (typically having a capacity of 1-2 microliters per well). Furthermore, the microdroplets can be made and manipulated at a frequency of up to 104 s^{-1} (kHz), which is about 10^4 times faster than existing high throughput screening technologies (up to 100,000 assays per day, or ~1 s^{-1}), or more in some cases, as described herein. The small volume of the microdroplets means that even proteins expressed from single genes or single cells can be analyzed. This reduction in the assay volume should also give large cost savings.

[0182] Cells (e.g., mammalian, yeast, bacteria, etc.) can secrete a variety of molecules (e.g. proteins, peptides, antibodies, haptens) that can be screened. The target molecules to be determined can also be produced, for instance, by in vitro transcription, in vitro translation (IVT), coupled in vitro transcription and translation, etc. of genes encapsulated in droplets. A signaling entity may be used to determine the target molecules. For instance, the signaling entity may include a binding partner of a target ligand or substrate for an expressed protein attached to the surface of a microparticle.

[0183] In some cases, prior to encapsulation, the binding partner can be coupled to the surface of a bead (e.g., a polymer bead, a microgel bead, etc.). In some embodiments, an antibody may be coupled to a bead using, for example, anti-antibody antibodies, protein A, protein G, protein L, and/or antibodies against an epitope tag on the expressed antibody. Depending on the application and the particular signaling entity used, the bead can be functionalized in an appropriate way in order to couple the sensor ligand to it (e.g. biotin-streptavidin link, epoxy-, carboxyl-, amino-, hydroxyl-, hydrazide-, chloromethyl-groups for proteins). Expressed proteins can bind to the binding partner, and/or catalyze the transformation of the binding partner on the bead (substrate) into a product. In other cases, the binding partner may be used to regulate the activity of another molecule co-encapsulated in the droplet so as to cause the binding partner to be bound by a ligand or transformed into a product.

[0184] The binding of the expressed protein to the signaling entity on the bead can be detected, as this example illustrates, by coencapsulation of a fluorescently labeled antibody which binds to the expressed protein (for example via an epitope tag). Other examples of fluorescent labeling include, but are not limited to, for example, fusion to a fluorescent protein such as GFP and/or fusion to a CCPGCC (SEQ ID NO: 1)